Antimicrobial resistance and mechanism of action of antimicrobial resistance

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Definition of antimicrobial agents

Antimicrobial agents are chemical substances (drugs) that has the capacity to reduce or eliminate the growth of microorganism (CDC,2008). The inhibitory or killing effects of antibiotics against bacterial to which it is initially sensitive and effective to is termed antibiotic resistance (Lee *et al.,* 2009). The change in the genetic constituent of an organism can be responsible for intrinsic resistant while resistance could also be due to natural susceptibility (Forbes *et al.,* 2002).

1. Emergency of Antibiotic Resistance

a) Dramatic success was recorded after the world war when antibiotics were introduced due to bacterial infections. Presently, antibiotics have lost this unique gain recorded as evident in treatment failure due to emergence of antibiotic resistance (Bud, 2007; Song *et al*., 2005). The sustainability of antibiotic resistance is due largely to selection pressure of already resistant bacteria that become the new dominant population in the environment. The increase in the use of antibiotics encouraged bacterial previously sensitive to antibiotics to become resistant in order to survive. The ability of these bacterial to survive is dependent on certain strategic mechanisms among which are alteration of the bacterial surface, mutations in specific gene sites and acquisition of antibiotic resistance genes from other bacterial (horizontal gene transfer). The excessive use and accumulation of antibiotics in the environment has resulted in the increase of antibiotics resistant bacteria in Nigeria and other continents of the world.

Several factors are responsible for the development of antibiotic resistance one of which is its misuse. Antibiotic use is influenced by important factors like level of information, choice of prescription, patient attitude, economic condition and the health system (McNuity and Francis, 2010). The patient factors may include inappropriate antibiotic use, like self-medication or inadequate doses despite the prevalence of self-medication. In Europe in 2006 by Grigoryan et al. (2006), it was observed that there were high level of self-medication in Eastern and Southern Europe than in Northern and Western Europe. The prescription system for drugs is very important. In developing countries, antibiotics can be purchase over the counter and prescription is not even necessary. Moreso, the antibiotics are bought in single doses, which increases the risk of the antibiotic treatment not being successful since treatment is often terminated before clinical success. It has also been observed that there has been an increased focus on persistent bacterial biofilm formation on medical devices implants and environmental biofilms (Hiby *et al.,* 2010; xu *et al.,* 2000). Amazingly, biofilms were shown to be very critical for horizontal gene transfer (Sorensen *et al.,* 2005) thereby facilitating the development of antibiotic resistance in bacterial.

Modern life style had led to increase in ageing in human and animal populations and in increased usage of antibiotics. Intensively managed patient in the hospital environment has led to new infections that are difficult to eradicate. Increased usage of broad spectrum antibiotics in order to avoid treatment failure resulted in vicious circle in the clinical environment as the broad spectrum antibiotics used have an influence on the level of multi resistant bacteria and their presence (Moller and Hansen, 2007).

b) The Gastrointestinal Tract as pool of antibiotic resistance Genes

The intestine whether animal or human is a complex system which accommodates large species of microorganisms (Borriello, 1986). Depending on the section of the gastrointestinal tract, large volume of bacteria 104 bacteria/ml in stomach to 1012 bacteria/g faces in the distal part of the colon (Zoetendal *et al.,* 2004). It has been reported by Amanm et al. (1995) using the classical culture techniques that about 5-15% of the species presented in the GI tract are detected.

However, new method using meta-genomic approaches of the bacteria flora suggested that bacterial species present may be as high as 1150 bacteria/ml (Qin and Racs, 2010). Several attempts have been made to control antibiotic resistance development but the success is minimal.

It is known that genes responsible for antibiotic resistance are resident in microorganisms, providing them with self-protection to the antibiotic compounds they produce as defense mechanism against other microorganisms. There exist some similarities between and among the genes and resistance mechanisms found in the antibiotic producers and in the animal/human pathogenic bacteria suggestive that the producer bacterial are the pool of origin of antibiotic resistance genes (Aarestrup *et al.,* 2000).

In the course of antibiotic treatment, virtually all the bacteria in the animal/human body are prone to selective pressure of the antibiotic. As a result, the gastrointestinal tract which is the field of bacteria colonization is highly exposed more especially during oral therapy. Hence, selection pressure due to naturally resistance strains carrying an important genetic pool which may have the ability of transferring antibiotic resistance genes to other strains present in the intestine of human/animal may result. Also, resistant food contaminants that originate from animals and are consumed by humans, can also act as a gene pool of antibiotic resistance genes (Andersson and Levin, 21999).

Resistance gene from both Gram-positive and Gram negative bacteria had shown similar sequences, an indication that transfer of antibiotic resistance genes across genera has occurred. It is pined that evolution of transfer events which has occurred may be as a result of high gene sequence similarity (Couvalin, 2008). Also, gene flux occurs naturally from Gram positive cocci such as enterococci to Gram-negative bacteria with genes coding for streptogramins as examples (Courvalin, 2008).

c) Lactic Acid bacteria: An intermediate hosts of Antibiotic resistance Genes.

Most food production is associated with involvement of microbial fermentation using lactic acid bacteria (LAB) strain (Boyle *et al.,* 2006), such foods as dairy products, cheese, and sausage. Also, the availability of probiotic in the open market containing either single strain or combination of strains. The impending danger is that both starter cultures and probiotics might contain naturally occurring antibiotic resistance genes which could be transferable. Increase in the level of consumption of fermented foods as well as increase in the level of interests in probiotic products may lead to gastrointestinal tract involvement. With increase in the level of Gram positive bacteria ingested, the possibility of these bacteria contributing to the pool of antibiotic resistance genes arises especially when the environment is conductive, these genes could be transferred to the endogenous flora. These antibiotic resistance genes when transferred to a bacteria that is pathogenic could lead to treatment failure of an infection.

d) Antibiotic Resistance Transfer to the indigenous flora in the Gut.

The postulation that the indigenous flora can become a reservoir of antibiotic resistance genes is yet to be proven; however, the suggestion that Gram-negative bacteria part of the flora has a greater possibility to obtain antibiotic resistance genes and might serve as a reservoir in transfer of resistance genes more to pathogenic bacteria resulting to infections with poor possibility of treatment (Tuohy et al, 2002). A good analogy is a hospital facility using more of antibiotics targeted at Gram-negative bacteria. A rat model involving human microbiotic showed that broad-host range plasmid p AMBI can be transferred from L. latics to indigenous enterococcus spp., whereas, there was no observable transfer to lactobacillus spp. and enterobacteriaceae spp (Burton *et al.,* 1974). In a study conducted in 1974 in human subjects the outcome revealed that antibiotic resistance occurred between an Escherichia in the gastrointestinal tract during tetracycline treatment. However, the transfer was observed late in the experiment (34 days after the treatment has ended. This development was with therapeutic levels of tetracycline 1000mg/daily) administered and not with the low level of 50mg/day (Lester,*et al.,* 2006).

In the study conducted by Balis et al. (1996), it was observed that vancomycin resistance has been transferred between enterococcus spp in human GIT. Also, the probability of transfer of a plasmid harbouring ampicilin resistance from the food contaminant salmonella of human is very possible and had been confirmed by in-vitro experiments of transfer at high frequencies. Antibiotic resistance gene transfer is of great concern, though transfer of extended genes is in a classification of its own, and this may lead to limitations in treatment (Cavaco, *et al.,* 2008).

It is however amazing that indigenous *E-coli* strains with a bla CTX-M ESBL gene were isolated from pigs. In the course of treatment with cephalosporins, the diversity of the indigenous *E.coli* strains receiving the *bla* CTX-M genes increased, suggesting horizontal gene transfer during selective pressure (Bidet *et al.,* 2005).

e) Antibiotic resistance in farm produce

Bacterial are present in micro-ecological niches, but more between ecosystems from animal to humans, from humans and animals (fallers and manure) to water and soil and return to human and animals via food (plants or vegetables).

Also, usage of antibiotic for treatment in each small niche (plants, animals and human) selects the resistant strains to become the reservoir of resistance genes. The antibiotic resistance genes are present and can be transported with the bacteria from one niche to another (Anonymous, 2006). Humans eat and ingest animal proteins and plants which might contain bacteria with antibiotic resistance genes, but the increase in consumption pattern of fermented food products increases the associated risk of antibiotic resistance genes to occur in the gastrointestinal tract.

Generally, human and animal foodstuffs contain bacteria which harbor antibiotic resistance genes, though pathogens that contaminate food is the major focus. Epidemiological studies have shown that through trace back, an outbreak of strain can be associated with specific food products Ethelberg et al. (2007), for example, the outbreak caused by *Salmonella* Kedougou Guardabassi et al. (2007), *Shigella Sonnei* infections caused by imported baby corn Emberland et al, (2006) and the presence of multi resistant *Salmonella* Typhimurium in Carpaccio Lewis et al. (2007).

Studies have shown that antibiotic resistance genes of different lactic acid bacteria species such as *Streptococcus thermophilus*, *Lactococcus spp*, and *Pseudomonas spp* are present in products such as cheese, meat products like raw milk, port chop, beef, and turkey (Klare *et a.l*, 2007). Studies conducted invitro has shown that Lactococcus lacks DNA containing tetracycline resistance gene tets were successfully transformed into the oral Cariogenic pathogen streptococcus mutants Klare et al, (2007). Though the studies revealed antibiotic resistant genes transfer from a commensal to a pathogenic bacterium, these events are yet to be confirmed in-vitro.

Antibiotic resistance genes transfer from Gram-positive to Gram-negative bacteria in-vitro is an uncommon event. The transfer of naturally occurring Gram positive plasmid p1p501 in-vitro in *E. faccalis* to *E. coli* has been described Charpenter et al. (1999). Also, naturally occurring plasmid, pip 823 from *Listeria monocytogenes*, was transferred only in the presence of the broad host range plasmid, PAMBI which initiate the transfer of both *E. faccalis* and *E. coli* Bertram et al. (1991). However, it is difficult for the opposite transfer situation because of the difference in the gene expression mechanism (Courvalin, 2008.).

2. Mechanism of antibiotic resistances in Salmonella

The mechanisms for antibiotic resistance can be classified into four groups; namely (a) modification or destruction of the antimicrobial agents, (b) using efflux mechanism to pump the antimicrobial agent from the cell (c) replacement of the antibiotic target, and (d) decrease in the cell permeability membrane. The acquiring of mobile genetic elements carrying resistance genes such as integrons, plasmid and transposons are the means by which microorganism develop resistance mechanisms (Walsh, 2003).

There are many classes of antimicrobial drugs but for the purpose of this study, the most common antimicrobials that Salmonella has developed resistance at present will be reviewed. These are: the aminoglycosides, B-lactans, chloramphenicol, quinolones, Sulfonamides/Trimethoprim and tetracycline.

a) Aminoglycosides

The aminoglycosides is active and effective against Gram negative bacteria. Due to its broad spectrum activity, it can be used in combination with other antibiotics (Gonzalez and Spencer, 1998). This combination may serve the sequence with 16SrRNA subunit of the 30s ribosomal Condon and translation inhibition of the formed binding. Both bacteriostatic and bactericidal activity is exhibited by most aminoglycosides bacteria. There are three mechanisms of resistance to aminoglycosides by bacteria and these includes, decrease permeability, alteration of ribosomal binding sites and antibiotic modifications, in other enterobacteria such as *E. coli,* the resistance to am aminoglycosides is by efflux pumps which takes out antibiotic within the cell (Aires and Nikaido, 2005). This mechanism does not play any significant role in salmonella aminoglycosides resistance, but facilitates its defence against other antibiotics. *Salmonella* uses mechanisms such as expression of plasmid-mediated aminoglycosides modifying enzymes against aminoglycosides (Guerra, 2002). These enzymes are classified into three groups namely; acetyltransferases, phosphotransferase and nucleotidyltransferases according to the reactions they perform.

b) Beta-lactams

These are made of the penicillins derivative namely; carbapenems, cephalosporins and monobactams (Petri, 2006). The mechanism of action of these groups is by interfering with penicillin-binding proteins (P_BP_s) with a group of seven proteins. The synthesis of peptidoglycan is facilitated by these proteins. This peptidoglycan is a very important component of the bacterial cell wall. Beta lactams are bactericidal in action even though the activity varies among the betalactams, organisms and target $P_BP_{s.}$ many organisms are becoming resistant to ampicilin and methicillin due to indiscriminate clinical use (Angulo, *et al.,* 2000).

Cephalosporin are second class of beta lactams with 6 member beta-latam ring rather than 5 and this provides cephalosporins with high level of efficiency and stability in the present beta-lactamases (Hornish and Kotarski, 2000). The carbapenem is the last group of beta-lactam which is very effective against gram positive and gram negative bacteria than other beta-lactam family, and diffuses easily in bacteria hence considered as broad spectrum B-lactam antibiotic. For this reason, their clinical uses are reserve for multi-drug resistance bacteria. There are indications of resistance to carbapenerns such as Imipenem by *Salmonella species* (Mariaguo *et al.,* 2003). The beta-lactam gets to the bacteria cell wall getting to their target PBP by using two porins OmpC and OmpF which catalyze the passage (Jaffe *et al.,* 1982). Previous studies have revealed that decreases in either OmpF or OmpC porin levels have generated an increase in resistance (Bellido *et al.,* 1989). Other reports show decrease in porin contents found that the reduction in

OmpF and OmpC porin expression lead to decreased resistance to most beta-lactams beside mecillina and imipenem in Salmonella envB mutants, this is due to other effect of env B mutation on the organism (Oppezzo *et al.,* 1991).

c) Chloramphenicol

It is a specific and potent protein inhibitor and accomplishes this by binding to the peptidyltransferase center of the 50s ribosomal units, thereby preventing peptide bond formation (Mascaretti, 2003). As a result of the binding to enzymes the drugs will prevent elongation of the peptides.

Chloramphenicol has a broad spectrum activity against gram positive and gram negative bacteria and its effectivity is due to its ability to cross the blood brain barriers hence a drug of choice for systemic infection. The use of chloramphenicol both in veterinary and human medicine for the treatment of salmonellosis for a long time has resulted in resistance strains. Two mechanisms are employed in which Salmonella resistance to chloroamphenicol is conferred namely; (a) plasmid located enzymes called chloramphenicol acetyltransferases (CAT) (b)Efflux pump in which the antibiotic is removed.

Salmonella Typhi isolates have been found to encode genes for *CAT* and are plasmid borne (Guerra et al, 2000). The *CAT* genes, such as *cat 1* and *cat 2* have also been found in Salmonella serotypes such as Derby, Enteritidis and typhimurium (Chen *et al.,* 2004). There are various Salmonella serotypes that have been found to carry *flor* genes, this gene are found in Salmonella genomic due to high mobility and close association with multi-drug resistance likely due to presence of plasmids carrying multiple resistance genes. (weill *et al.,* 2005; Alcaine *et al.,* 2005).

d) Quinolones

There are many generations of quinolones effective against bacterial infection. Their mode of action may vary depending on whether it is the early or late generation of quinolones targeting DNA gyrase and DNA topoisomerase, but to the double stranded DNA in the topoisomerase complex (Shen and Pernet, 1985).

There are reports of *Salmonella* with level of resistance to other quinolones (Brevil *et al.,* 2000).
Salmonella resistance to quinolone has been classified into two mechanisms. First is the two *gyr A* and *gyr B* genes which encode for the subunits of DNA gyrase and will be targeting mutations in the quinolones resistance-determining region (QRDR), and in the part C subunit of topoisomerase IV (Baucheron *et al.,* 2004). The second mechanism involves change in the *Acr AB- ToIC* efflux system expression, as a result of mutations in the regulator genes of this system due to over-expression of this efflux system (Levy *et al.,* 2004), which makes quinolones sensitivity to decrease.

In some bacteria organism like *E. coli* and *K. pneumonia* the expression of a plasmid mediated gene called *gnr* has also been associated to quinolone resistance (Li *et al.,* 2005). There, the gene expresses a protein that appears to bind to DNA-gyrase and protect it from quinolone inhibition (Li *et al.,* 2005). Studies on plasmids harbouring *gnr* show other bacteria species to salmonella (Martinez, et al, 2005). Other studies suggested that spend of such plasmids to salmonella has occurred and plasmid medicated quinolones resistances in Salmonella are not common (Cheunget *et al.,* 2005).

e) Sulfonamide & Trimethoprime

These classes of antibiotics are generally bacteriostatic and their mechanism of action is associated with inhibiting enzymes involved in the synthesis of tetrahydrofolic acid. The sulfonamides inhibit dihydropterote reductase (DHFR) (Mascaretti, 2003). The resistance of Salmonella to Sulfonamide has been attributed to the presence of an extra *sul 1, sul 2* and *sul 3* and they are the three main genes that have been identified. *Salmonella* Serotypes such as Hader, Enteritidis, Heidelberg Agona, Derby and typhimurium are known to harbor the *sul l* gene (Doublet et al, 2004).

The class 1 intergrons that contain other resistance gene have been associated with this gene. These integron gene cassettes have been located on transferable plasmids and as part of Salmonella genomic island variants (Boyd et al, 2002). The *sul 2* genes are harboured by many *Salmonella* serotypes including Agona, Enteritidis and typhimurrium isolates.

The *sul 3* gene is harboured by many salmonella serotype such as Agona, Bradenburg and Tymphimurium and is also known to be associated with plasmids, (Antunes *et al.,* 2005). Resistance to trimethoprim is attributed to the activity of DHFR. Common serotypes that have trimethoprim resistance genes are 4,5,12,:1, Agona, Djugu, Hader, Neport, Albany and Tymphimurium. These genes have been found as part of integron-borne gene cassettes also associated with *sul-1* and *sul 3* on transferrable plasmid carrying other resistance genes and *Salmonella* genomic island (Villa and Carratoli, 2005).

f) Tetracyclines

Tetracyclines are broad spectrum antibiotics effective against many gram negative bacteria (Mascaretti, 2003). The mechanism of action is by stopping the binding of tRNA to the "A. site of the 30s ribosomal submit by inhibiting protein synthesis (Mascaretti, 2003). Tetracycline use has been limited due to increase in antibiotic resistant bacteria. Studies suggest that tetracycline resistance in *Salmonella* may be associated with production of an energy dependent efflux pump to remove the antibiotic from within the cell. The modification of the ribosomal target, enzymatic

inactivation of tetracycline and other mechanism of resistance, have been documented in other bacteria species but yet to be reported in Salmonella (Chora and Roberts, 2001).

In *Salmonella*, different tetracycline *(tet)* genes have been discovered to conferring resistance to tetracycline in Salmonella Serotypes. The common types of *tet* genes belong to the classes A,B,C,D and G (Chopra and Roberts, 2001). Thus, the *tet*(*G)* genes was found in salmonella genomic island 1, the *tet (A)* gene was found on plasmids and chromosome, while *test (B), tet (D)* genes were located on the chromosomes of many Salmonella enteric serotypes, including Saintpaul, Enteritidis, Hader, Infantis, Derby, Colorado and Heidelberg (Pezella et al, 2004).

The *tet (A) and tet (B)* gene are found in transferable plasmids and these genes are easily transferred and are wide spread among *Salmonella.* Most of these isolate are found to have multidrug resistance making them an important identification marker in identifying potentially Salmonella infection (Pezzella *et al.,* 2004).

3. Conclusion

The increasing use of antibiotics over a period of time without recourse to prescription and effective antimicrobial sensitivity test has led to the emergence of resistance. The mechanisms of action of these antibiotics vary depending on the class of antibiotics in question. Mechanisms ranging from decrease in permeability to alteration of ribosomal binding sites and antibiotic modification. Effort should be made to reduce the misuse of antibiotics in order to limit the rate of emergence of resistance to these drugs

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